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Characterization of commercial soybean products by conventional and perfusion reversed-phase high-performance liquid chromatography and multivariate analysis

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Abstract

Conventional and perfusion reversed-phase high-performance liquid chromatography are used to characterize commercial soybean products for human consumption. For this purpose, previously optimized methods of conventional and perfusion chromatography applied to the separation of soybean proteins are employed. Sixty different samples corresponding to 26 different trademarks of soybean products [soybean protein isolate, soybean flour, textured soybean, soybean milks (liquid and powdered), and soybean infant formulas] are analyzed. Characterization of soybean products is carried out on the basis of their protein profiles obtained by both chromatographic methods. Data obtained are processed using multivariate methods such as principal components and discriminant analysis. Perfusion chromatography enables a further and faster characterization of commercial soybean products than conventional chromatography, of great value in the quality control of this kind of product.

Keywords: Soybean; Food analysis; Multivariate analysis; Perfusion chromatography; Proteins

1. Introduction

The increasing consumption of soybean products as a result of the interesting properties associated with the use of soybeans has promoted the appearance of a wide range of foodstuffs derived from this seed: soybean flour, textured soybean, soybean milks, soybean infant formulas, etc. [1–9]. Due to this huge diversity of products from soybean, their characterization could be very useful concerning the prediction of their origin and processing control.

The main proteins present in soybean (11S globulin or glycinin and 7S globulin or β-conglycinin) have been characterized by different techniques such as gel electrophoresis [10–15] and immunological methods (immunoblotting [11,15], immunoelectrophoresis and double gel immunodiffusion [16–18], and enzyme-linked immunosorbent assay [19,20]). High-performance liquid chromatography (HPLC) has also been applied to the characterization of soybean proteins, reversed-phase and size-exclusion being the main chromatographic modes which have been used [21–24]. Some authors have studied the potential of this technique for soybean cultivar identification [22,23,25,26]. However, with regard to commercial soybean products, our research team was the only one that has opti-

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mized methods of reversed-phase HPLC (RP-HPLC) [27] and capillary electrophoresis [28] to carry out the characterization of these products. In these works, the separation of soybean proteins from commercial soybean products such as basic products (products normally used as starting materials for the preparation of other more elaborated ones) and soybean dairy-like products has been carried out.

Although the application of HPLC to the separation of soybean proteins has implied an important reduction of analysis times, new stationary phases have also provided further improvements. In this respect, perfusion chromatography, which uses polymeric packing materials having a bimodal structure of macroporous (6000–8000 Å) interconnected by a smaller-size diffusive porous structure (800–1500 Å), has enabled the reduction of the analysis times required to separate molecules such as proteins without significant losses in resolution, efficiency or capacity [29–34]. This technique has been recently applied to the quantitation of soybean proteins in commercial soybean products [35,36] and to the simultaneous separation of soybean and bovine whey proteins by our research team [37].

The aim of this work is to characterize commercial soybean products based on their protein profile obtained by conventional and perfusion reversed-phase chromatography. Part of the chromatographic profiles used were obtained in this work and part were taken from previous works [27,35,36]. All these data were processed using multivariate analysis.

2. Experimental

2.1. Chemicals and samples

HPLC-grade acetonitrile (ACN) (Scharlau, Barcelona, Spain), trifluoroacetic acid (TFA) (HPLC-grade; Pierce Europe, Ond-Beijerland, The Netherlands), and HPLC-grade water (Milli-Q system; Millipore, Bedford, MA, USA) were used in the preparation of the mobile phases.

Tris(hydroxymethyl)aminomethane and 2-mercaptoethanol (analysis grade, Merck, Darmstadt, Germany) were used for the fractionation of soybean proteins.

Sixty different commercial soybean products (soybean protein isolate, two soybean flours, three textured soybeans, six powdered soybean milks, 26 liquid soybean milks, and 22 soybean infant formulas) corresponding to 26 different trademarks have been analyzed. All these products were purchased from local markets and chemists of Alcalá de Henares, Madrid, Spain. On the other hand, the soybean protein isolate (SPI) was obtained from ICN (Aurora, OH, USA).

11S and 7S globulin fractions were obtained from either soybean protein isolate, soybean flour, textured soybean or three powdered milks using the method of Thanh and Shibasaki [38].

The protocol for preparing solutions of soybean products was the following [35]: the sample was weighed and dissolved in water, then the mixture was sonicated for 3 min and centrifuged (3000 rpm, 5 min, 3°C; Jouan, Saint Herblain, France) to remove the supernatant which was kept on ice until its injection into the chromatographic system.

2.2. High-performance liquid chromatography

A Hewlett-Packard 1090 Series II liquid chromatograph (Hewlett-Packard, Pittsburgh, PA, USA) equipped with a diode-array detector and a HP 9153C data acquisition system was used. The injection volume was 20 μl.

All peak area percentages obtained in the separation of soybean proteins by conventional RP-HPLC were taken from a previous work [27] in which a 8-μm particle size (300 Å pore size) PLRP-S (Polymer Labs, Church Stretton, UK) column (150×4.6 mm I.D.) was used. Peak area percentages obtained in the separation of soybean proteins from commercial soybean products by perfusion RP-HPLC were taken from Refs. [35,36]. These data were obtained using a 10-μm particle size POROS R2/H (PerSeptive Biosystems, Framingham, MA, USA) column (50×4.6 mm I.D.). Finally, data corresponding to peak area percentages for the separation of soybean proteins from globulin fractions by perfusion RP-HPLC have not been published before, and were obtained when using the same perfusion column described above.

The experimental conditions used for separating soybean proteins by conventional RP-HPLC were those optimized by García et al. [27] (flow-rate, 1 ml min^{-1}; temperature, 50°C; detection wavelength, 254 nm). The elution was performed with a linear binary gradient at 20% B for 1 min, 20–35% B for 19 min, and 35–46% B in 0.5 min, followed by a linear gradient from 46% to 20% B in 0.5 min to re-equilibrate the column. The experimental conditions in perfusion RP-HPLC were those optimized by García et al. [35]: flow-rate, 3 ml min^{-1}; temperature, 60°C; detection wavelength, 254 nm. Soybean proteins were eluted in a linear binary gradient (5–25% B in 1.7 min and 25–45% B in 1.3 min). A linear gradient from 45% to 5% B (1 min) was performed between runs to re-equilibrate the column. Two mobile phases (A and B) were used: A, 0.1% (v/v) TFA in water; B, 0.1% (v/v) TFA in ACN. Mobile phases were filtered using 0.45-μm nylon filters and degassed with helium before use.

2.3. Data treatment

Peak areas corresponding to soybean proteins were integrated by setting the baseline from valley to valley. The area percentage for every peak was calculated as the average of three area percentages corresponding to three different injections of each sample solution.

Box-and-whisker plots were used to compare different batches of data. This plot was performed by using the Statgraphics Plus program [39]. Application of multivariate methods has been carried out using the Statgraphics Plus program [39].

3. Results and discussion

3.1. Chromatographic profiles obtained for commercial soybean products

In a previous work, the chromatographic profiles obtained for some soybean commercial products by conventional RP-HPLC were studied by our research team [27]. In that work it could be observed that chromatographic profiles of basic products such as soybean protein isolate, soybean flour, and textured soybean differed from those obtained for dairy-like products [soybean milks (liquid and powdered) and infant formulas]. In fact, basic products presented chromatograms with five peaks, while in chromatograms corresponding to dairy-like soybean products, peak 3 did not appear. Fig. 1 shows, as an example, the chromatograms corresponding to two basic products (soybean protein isolate and soybean flour) and to a soybean infant formula obtained using conventional RP-HPLC. As observed, peak 3 appears in the soybean protein isolate and soybean flour chromatograms while it does not appear in the soybean infant formula.

A chromatographic method using perfusion RP-HPLC was recently optimized for separating soybean proteins from soybean protein isolate in eight peaks, in less than 3 min [35,36]. When this perfusion method was applied to the separation of soybean proteins from commercial soybean products, differences in the chromatographic profiles corresponding to different kinds of products (powdered milks, liquid milks or infant formulas) were found. In addition, products derived from soybean protein isolate presented different chromatographic profiles from those prepared directly from whole soybeans (WS). In fact, chromatograms of samples of powdered milks from soybean protein isolate presented a higher number of peaks than those obtained with samples of powdered milks from whole soybeans. Fig. 2 shows the chromatogram corresponding to a powdered soybean milk prepared from soybean protein isolate in which the presence of eight peaks is observed and the chromatogram corresponding to a powdered soybean milk prepared directly from whole soybeans in which peaks 7 and 8 disappear.

Regarding liquid soybean milks, the following samples were examined: six elaborated from soybean protein isolate (corresponding to two different trademarks), three from a protein extract from an ecological soybean cultivar (corresponding to the same trademark), and 17 from whole soybeans. All chromatograms obtained for these liquid milks showed peaks 1, 3 and 8, while peaks 2, 4–7 might or might not appear based on the trademark or milk lot. In this case, due to the huge number of different chromatographic profiles obtained, the differentiation among soybean liquid milks prepared from different starting materials was not as clear as in the case of the powdered soybean milks.

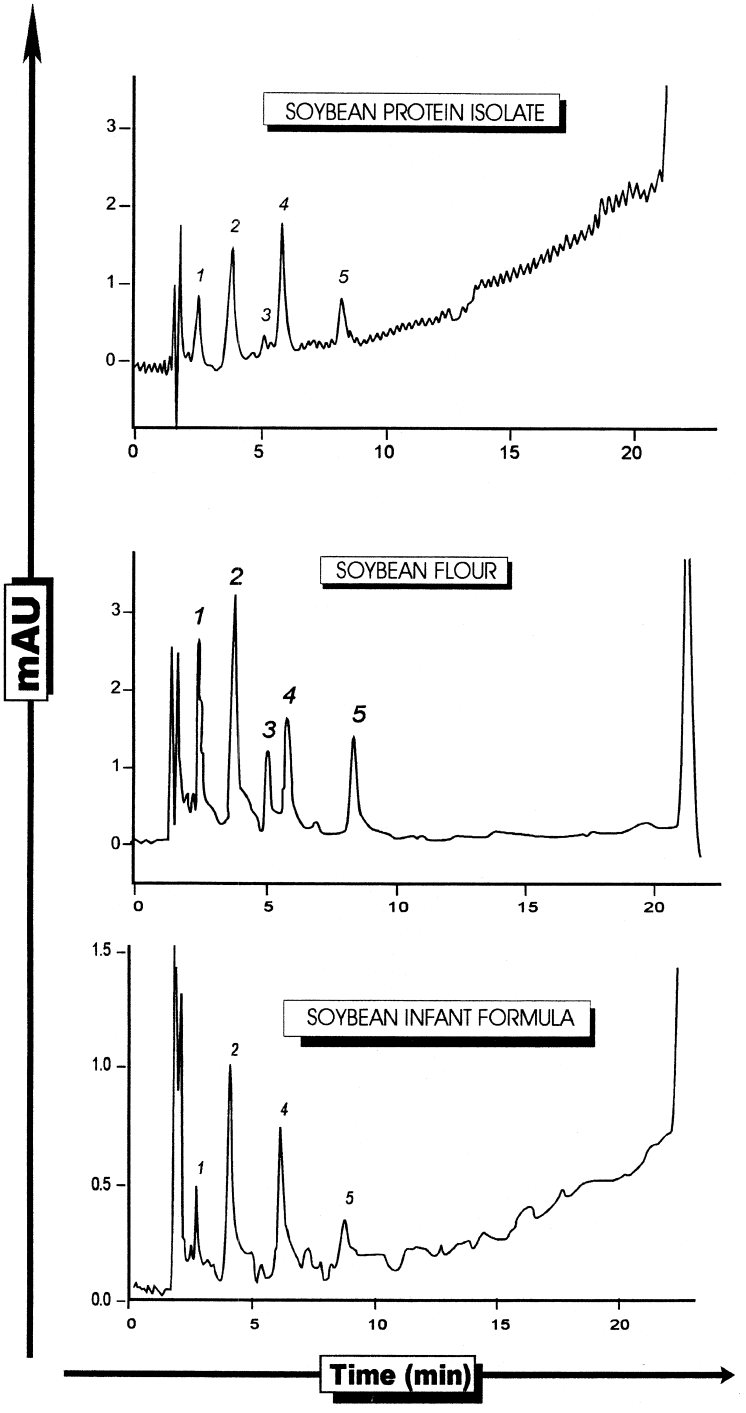

Fig. 1. Chromatograms corresponding to aqueous solutions of soybean protein isolate (0.92 mg ml^{-1}), soybean flour (0.92 mg ml^{-1}) and soybean infant formula (3.62 mg ml^{-1}) (all as dry basis) by conventional RP-HPLC. Experimental conditions: flow-rate, 1 ml min^{-1}; temperature, 50°C; detection, 254 nm; gradient: 20% B for 1 min, 30–35% B in 19 min, and 35–46% B in 0.5 min; mobile phases: A, 0.1% (v/v) TFA in water; B, 0.1% (v/v) TFA in ACN.

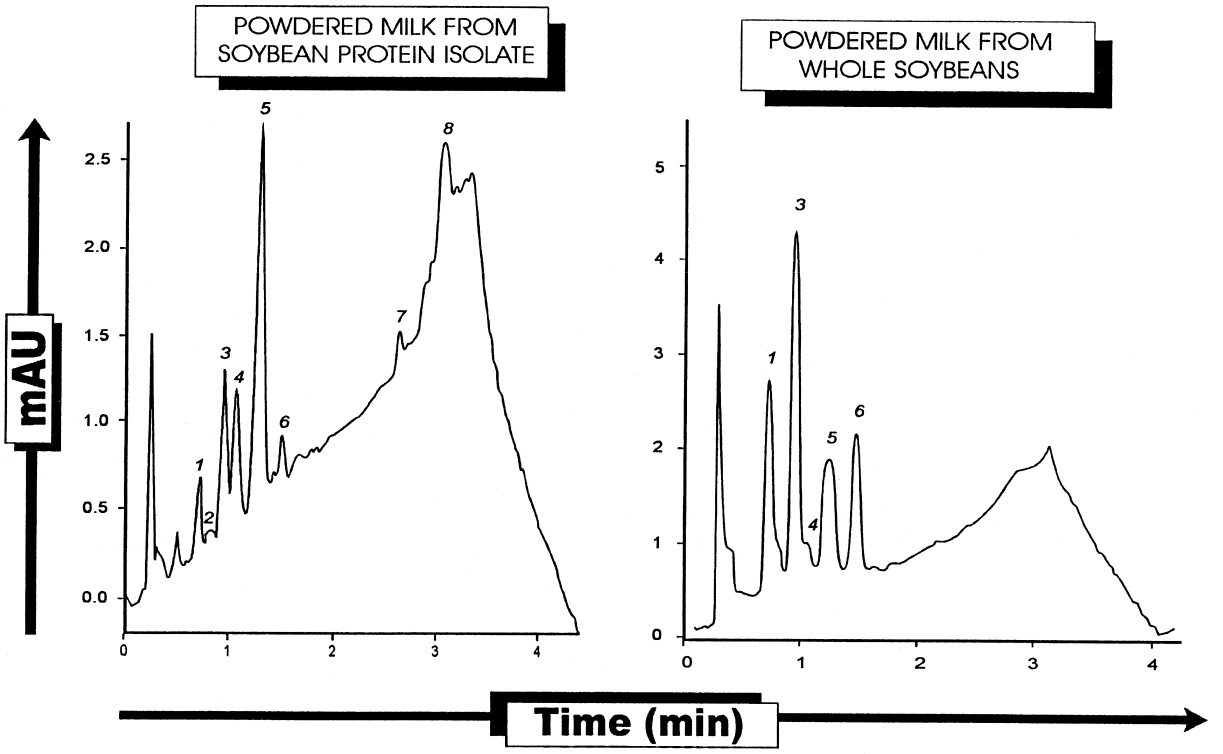

Fig. 2. Perfusion RP-HPLC separations of soybean proteins from aqueous solutions of a powdered soybean milk from a soybean protein isolate (1.76 mg ml^{-1}) and a powdered soybean milk from whole soybeans (1.20 mg ml^{-1}) (both as dry basis). Experimental conditions: flow-rate, 3 ml min^{-1}; temperature, 60°C; detection, 254 nm; gradient: 5–25% B in 1.7 min and 25–45% B in 1.3 min; mobile phases: A, 0.1% (v/v) TFA in water; B, 0.1% (v/v) TFA in ACN.

All soybean infant formulas are prepared from soybean protein isolates, and therefore their chromatographic profiles should be similar. Nevertheless, two different behaviours could be observed. In most cases, they presented chromatograms in which peaks 1–6 appeared, while for a minor group of soybean infant formulas, chromatograms showed only peaks 1, 3, 5 and 6.

In addition to the differences found among the chromatographic profiles obtained for soybean products, the relative size of each chromatographic peak in the different samples studied was also different. Thus, area percentages of every peak in all soybean products were grouped according to the type of product (liquid milks, powdered milks or infant formulas) and the raw material used in its preparation (soybean protein isolate or whole soybeans) and presented in box-and-whisker plots (Fig. 3). According to the diagrams, regardless of the raw material used, peak 2 was always a minor peak in the products studied. Among products prepared from soybean protein isolate, the predominant peak was peak 5, except for liquid milks, for which the predominant peaks were 3 (35–42%) and 8 (31–36%). Related to those soybean products prepared from whole soybeans, peak 3 was the predominant in all cases, and peaks 7 and 8, which did not appear in powdered soybean milks, exhibited a high variability (0.1–15%) in the liquid milks.

The chromatographic analysis of protein fractions enriched in 11S and 7S globulins was performed to assign chromatographic peaks to specific soybean proteins such as 7S and 11S globulins [1]. Globulin fractions were obtained from soybean protein isolate, soybean flour, textured soybean, and powdered soybean milks. Fig. 4 shows the 11S and 7S globulin chromatograms obtained from a powdered soybean milk prepared from a soybean protein isolate when

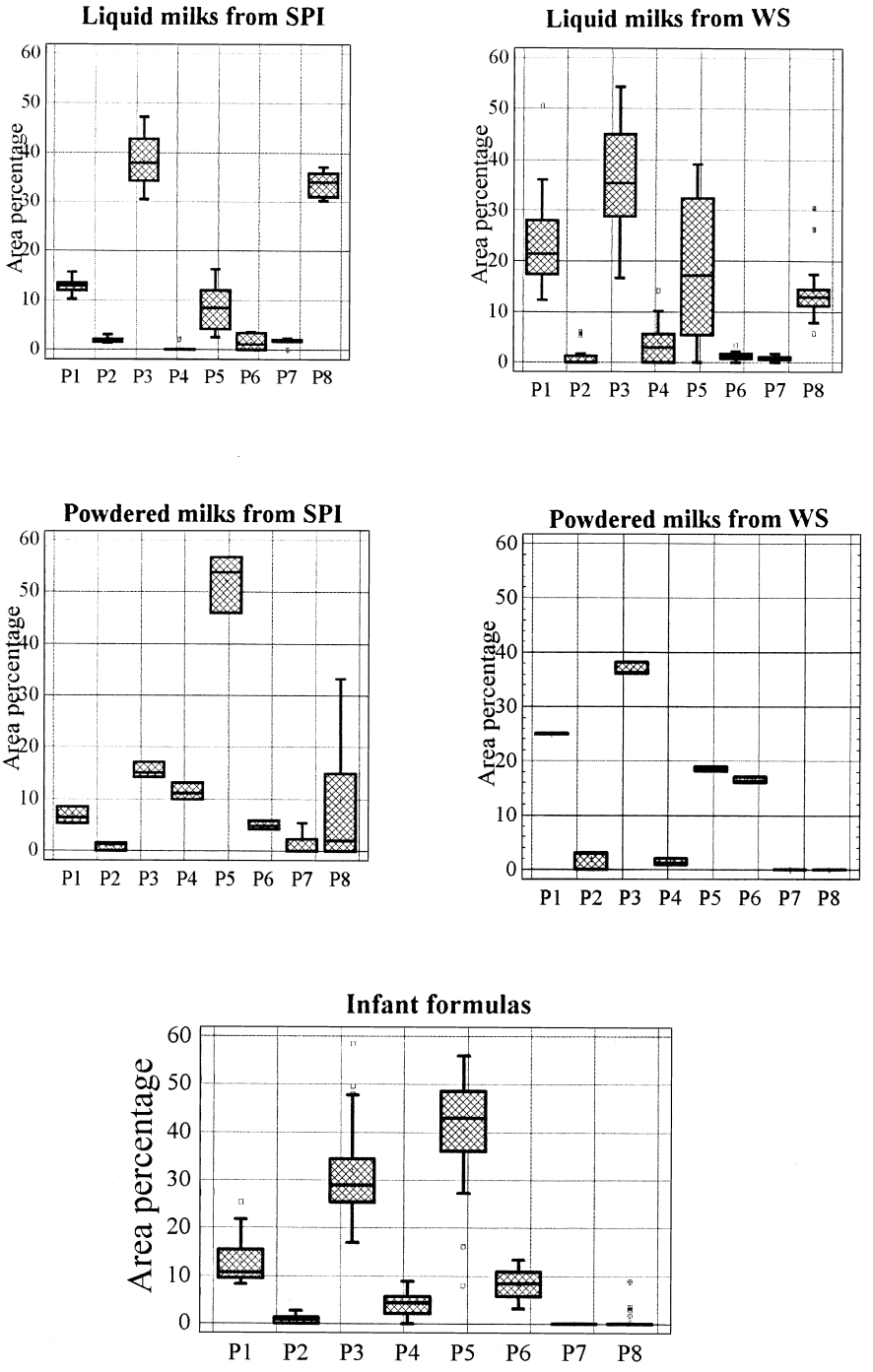

Fig. 3. Box-and-whisker plots for the area percentages of every peak (P1, P2, ...) obtained by perfusion RP-HPLC for liquid and powdered milks prepared from a soybean protein isolate (SPI) and whole soybeans (WS) and for soybean infant formulas. Data taken from Refs. [35and36].

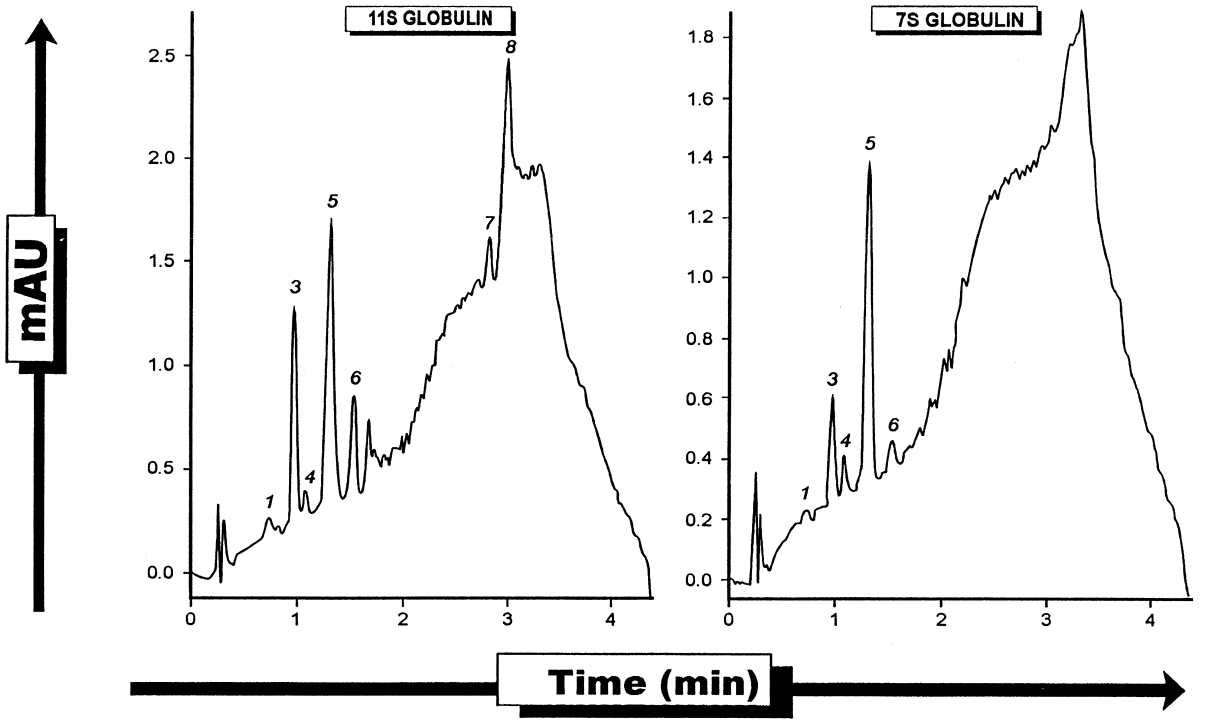

Fig. 4. Perfusion RP-HPLC separations of soybean proteins from aqueous solutions of 11S (3.00 mg ml^{-1}) and 7S (2.00 mg ml^{-1}) globulin fractions isolated from a powdered soybean milk prepared from a soybean protein isolate. Experimental conditions as in Fig. 2.

using perfusion RP-HPLC. The 11S globulin chromatogram is similar to that obtained for the powdered soybean milk (Fig. 2) whereas in the chromatogram corresponding to the 7S globulin, peaks 7 and 8 do not appear. This behaviour could also be observed in the fractions isolated from other soybean products. Table 1 groups area percentages for every peak in the 11S and 7S globulins and in the samples from which they were obtained. By comparison of these area percentages, it could be observed that peaks appearing at the beginning of the chromatograms seemed to contain a major ratio of 7S globulin while peaks appearing at the end of the chromatograms seemed to present a higher content of 11S globulin.

When analyzing globulin fractions using conventional RP-HPLC [27], it was observed that 11S and 7S globulin presented similar chromatograms, which were, at the same time, similar to the chromatogram corresponding to the original product (from which they were isolated). Nevertheless, examining area percentages for every peak in the globulin fractions and in the products from which they were prepared, it was observed that, as in perfusion chromatography, peaks at the beginning of the chromatogram seemed to be enriched in the 7S globulin whereas peaks at the end of the chromatogram were enriched in the 11S globulin.

3.2. Multivariate analysis

Multivariate analysis, mainly principal components and discriminant analysis, was applied to the area percentages of peaks obtained for the different soybean products studied when using either conventional or perfusion RP-HPLC [27,35,36]. Concerning conventional RP-HPLC, principal components analysis enabled the reduction of the five original variables, corresponding to the five peak area percentages, to three, which account for 97% of the variability of the original data. These new variables enabled the classification of the soybean products

Table 1
Area percentages of peaks 1–8 obtained by perfusion RP-HPLC for soybean protein isolate, soybean flour, textured soybean, and three powdered soybean milks and their corresponding proteic fractions[a]

Sample	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8
Soybean protein isolate	2.78	0.70	8.43	6.48	40.58	2.67	3.46	34.60
Fraction 11S	5.41	–	21.86	6.93	44.64	12.10	–	9.04
Fraction 7S	4.28	–	22.11	9.74	53.12	10.75	–	–
Soybean flour	16.55	1.61	25.58	8.27	30.88	12.42	0.42	4.10
Fraction 11S	6.18[b]		14.07[c]		19.04	14.07	3.51	43.20
Fraction 7S	10.36[b]		33.13[c]		38.54	17.96	–	–
Textured soybean	15.85	0.95	25.24	8.76	35.10	14.09	–	–
Fraction 7S	9.56[b]		32.06[c]		38.70	15.28	–	4.38
Powdered soybean milk A[d]	8.60	–	14.27	9.94	53.77	5.89	5.46	2.06
Fraction 11S	–	–	–	8.31	72.71	11.86	–	9.41
Fraction 7S	2.99	–	14.05	9.83	65.43	7.68	–	–
Powdered soybean milk B[d]	5.26	1.22	15.13	11.21	45.99	4.21	2.44	14.95
Fraction 11S	2.01	0.39	20.86	2.22	42.13	11.88	3.17	20.51
Fraction 7S	2.67	0.90	12.57	6.35	75.61	3.28	–	–
Powdered soybean milk C[e]	24.94[b]		38.09	2.03	18.89	16.04	–	–
Fraction 11S	14.28	–	41.46	–	18.45	24.84	–	–
Fraction 7S	14.28	1.84	44.33	1.75	21.73	16.06	–	–

[a] The number of chromatograms obtained in the same day for every sample was three.
[b] Area percentage corresponding to peaks 1+2.
[c] Area percentage corresponding to peaks 3+4.
[d] Powdered soybean milk from soybean protein isolate.
[e] Powdered soybean milk from whole soybeans.

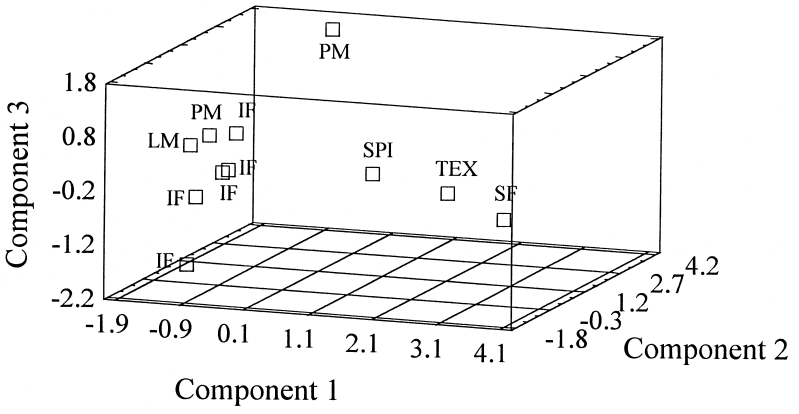

Fig. 5. Representation of the principal components obtained from peak area percentages for the soybean products studied [soybean protein isolate (SPI), soybean flour (SF), textured soybean (TS), liquid milk (LM), powdered milks (PM), and infant formulas (IF)] by conventional RP-HPLC.

studied into two different groups (Fig. 5), soybean dairy-like products and basic products. On the other hand, discriminant analysis required only one discriminant function to classify soybean products into the two previously established groups.

When applying principal components analysis to perfusion RP-HPLC chromatograms, the original eight variables corresponding to eight peak area percentages were reduced to three accounting for 81% of the original data variability. Due to the higher number and diversity of the soybean products studied, the representation of these new variables did not enable a clear distribution of soybean products into distinct groups. Nevertheless, when using discriminant analysis, two discriminant functions (97% of differentiation) allowed the classification of commercial soybean products into four groups: infant formulas, powdered milks, liquid milks, and basic products (Fig. 6). These results suggested that perfusion RP-HPLC enabled a better discrimination among soybean products than conventional RP-HPLC where soybean products were divided into dairy-like or basic products.

To obtain a deeper differentiation among soybean products, discriminant analysis was again applied to peak area percentages obtained using perfusion chromatography establishing more specific groups: infant formulas, powdered milks from soybean protein isolate, liquid milks from soybean protein isolate, liquid milks from whole soybeans, powdered milks from whole soybeans, and basic products. In this case, the number of discriminant functions found was five, two of which account for the largest differentiation (76%). The representation of these discriminant functions (Fig. 7) enabled the distribution of every soybean product studied into one of the six previously established groups, i.e. the differentiation is possible not only among kinds of soybean products (infant formulas, liquid milks, powdered milks, and basic products) but also among products prepared from different raw materials (whole soybeans or soybean protein isolate).

4. Conclusions

Conventional RP-HPLC enables the differentiation of soybean products into two different groups: dairy-like and basic products.

Perfusion RP-HPLC allows a greater differentiation: it is possible to discriminate among different kinds of soybean products (infant formulas, liquid milks, powdered milks, and basic products) and within every kind of product, between those prepared from soybean protein isolate and those directly elaborated from whole soybeans. This fact, together with the shorter analysis times needed to separate soybean proteins, make this perfusion method a suitable tool for the characterization of commercial soybean products on the basis of their chromatographic profiles.

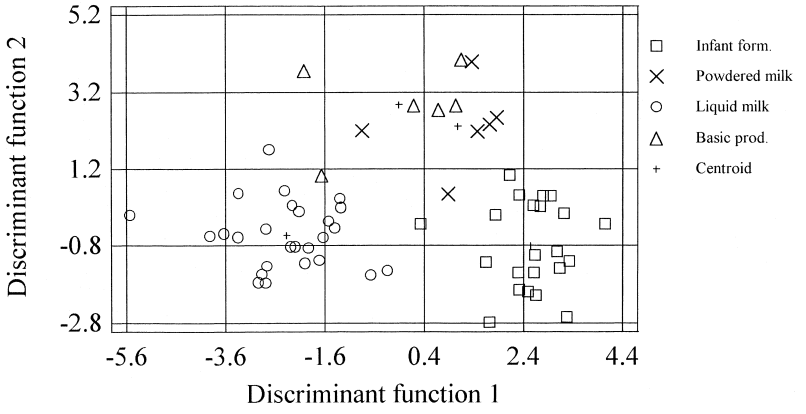

Fig. 6. Representation of the discriminant functions found from peak area percentages obtained by perfusion RP-HPLC for all soybean products studied (soybean protein isolate, soybean flour, textured soybean, powdered milks, liquid milks, and infant formulas) when establishing the following groups: infant formulas, powdered milks, liquid milks, and basic products.

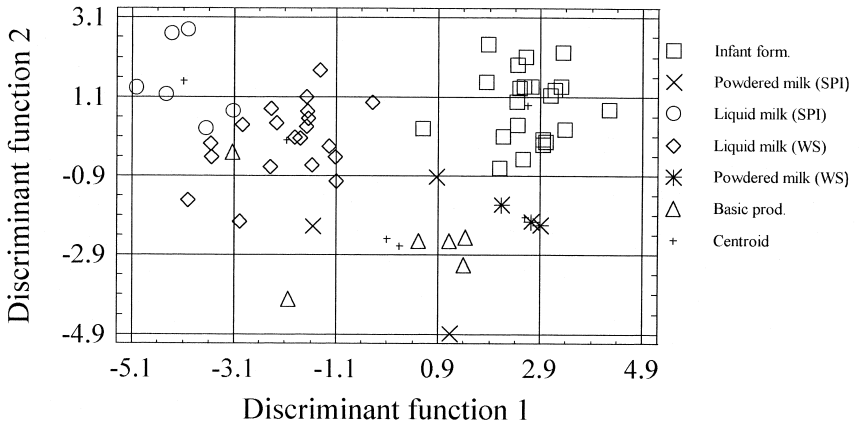

Fig. 7. Representation of the discriminant functions found from peak area percentages obtained by perfusion RP-HPLC for all soybean products studied (soybean protein isolate, soybean flour, textured soybean, powdered milks, liquid milks, and infant formulas) when establishing the following groups: infant formulas, powdered milks from soybean protein isolate (SPI), liquid milks from soybean protein isolate, liquid milks from whole soybeans (WS), powdered milks from whole soybeans, and basic products.

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